IARC Monographs are written by a Working Group (WG) over a period of about 12 months to evaluate all of the scientific literature on a given substance and, through a transparent and rigorous process[1], reach a decision on the degree to which the scientific literature supports the ability of that substance to cause cancer. For Monograph 112[2], 17 expert scientists evaluated the carcinogenic hazard for 4 insecticides and the herbicide glyphosate. The WG concluded that glyphosate was a probable human carcinogen. This finding stirred great debate globally on the safety of glyphosate and lead to a careful evaluation of the IARC monograph results when they came available on July 29, 2015. On August 31, 2015, the German Federal Institute for Risk Assessment (BfR) completed an addendum[3] (the BfR Addendum) to the Draft Renewal Assessment Report[4] (RAR) for glyphosate. This addendum was leaked by the media[5]. This letter is in response to the BfR Addendum and all signatories of this letters were members of the IARC WG for Monograph 112.

Our comments to the BfR Addendum will focus on the human evidence, the animal laboratory evidence and the mechanistic evidence.

The Human Evidence

The BfR agrees with the IARC WG that there is "limited evidence in humans for the carcinogenicity of glyphosate". In the IARC review process, this is defined as "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."[1] The BfR Addendum (p. ii) then characterizes the IARC interpretation as "precautionary" and takes a more "cautious view" of this classification because "no consistent positive association was observed", "the most powerful study showed no effect" and that the studies "could not differentiate between the effects of glyphosate and the co-formulants". We will consider the first two arguments here and third argument for the end of our letter.

The finding of "limited evidence" by the IARC WG was for non-Hodgkins lymphoma (NHL). When done correctly, cohort studies are very important in determining the carcinogenicity of an agent because they generally have less chance for bias, confounding and missclassification than case-control studies. The Agricultural Health Study (AHS) was the only cohort study available providing information on the carcinogenicity of glyphosate. The BfR refers to this study as "the most powerful study" and that it was negative for NHL. The study had a very weak positive finding for NHL (RR 1.1, 0.7-1.9) with no apparent exposure response in the results. Had this been the only study available, the WG would certainly not have classified glyphosate as "limited evidence".

The potential limitations with case-control studies can be found in any competent epidemiology textbook [6] and the BfR uses these to list all of the case-control studies as unreliable. This gives the impression that all of the studies are equal in quality and

unusable for an overall evaluation. This is not the case. An IARC WG carefully evaluates all of the available epidemiology data, looking at the study's strengths and weaknesses as well as the study order. This is key in determining whether the positive associations seen are a reliable indication of an association or simply a chance finding. Finally, the meta-analysis cited in the IARC Monograph[7] and redone by the WG is the best method for evaluating if there is a consistent positive trend; this meta-analysis showed a statistically significant association. The BfR concludes (p. 22) "there was no unequivocal evidence for a clear and strong association of NHL with glyphosate". We agree, but still consider that an association is observed, that causality is credible and that these findings should be used as part of the overall evaluation.

Evidence from Chronic Exposure Animal Studies

We are astonished by the conclusions of the BfR regarding the animal carcinogenicity data. In the IARC WG review, we found a significant positive trend for renal tumors in CD-1 mice[8] and since this was a rare tumor, compared it to an appropriate historical control dataset[9] for CD-1 mice demonstrating even greater significance. A significant positive trend means that as the exposure increases, the pattern seen in the data supports an increasing risk with increasing dose. There were no significant comparisons of any individual exposure group to the control group, however the high exposure group was highly significantly different the historical control population. We also identified a significant positive trend for hemangiosarcoma in male CD-1 mice[10], again with no individual exposure group significantly different from controls. Finally, we also saw a significant increase in the incidence of islet cell adenomas in two studies in Sprague-Dawley rats[11-13]. In one of these rat studies, thyroid adenomas in females and liver adenomas in males were also increased. Thus, glyphosate was positive for malignant tumors in both of the mice studies we examined and for benign tumors in 2 of the five rat studies we examined. By the IARC review criteria[1], the evidence in the mouse constitutes sufficient evidence in animals. The BfR agreed, stating (p. 44) "it is obvious that IARC concludes on "sufficient evidence of carcinogenicity" because the criteria for this conclusion are fully met."

It was clear at the time of our review that other studies had been done, but they were not publicly available in sufficient detail for independent scientific evaluation (a requirement of the IARC Preamble[1]). Based on the BfR Addendum, it seems there were 3 additional mouse studies and 2 additional rat studies where they had sufficient evidence to review the findings. Remarkably, the findings of these studies independently replicated the studies reported in the Monograph. BfR reported on two additional studies with a positive trend for renal tumors, one in CD-1 mice[14], and one in Swiss-Webster mice[15]. One of these studies[14] also reported a positive trend for hemangiosarcoma. Moreover, BfR reported two studies in CD-1 mice showing significant trends for malignant lymphoma[14, 16]. For all of the tumors described above in CD-1 mice, a positive trend was seen against the concurrent control.

However, in all cases in CD-1 mice, including those observed by the IARC, the BfR dismisses the observed tumors because there are no treatment groups which are significantly different from controls and because the maximum observed response is

within the range of the historical control data (Table 5.3-1 in the Addendum). Care must be taken in using historical control data to evaluate animal carcinogenicity data. In virtually all guidelines[17] and publications[18-20] on the issue, the first choice should be the use of the concurrent controls. For instance, the Preamble to the IARC Monographs states, "it is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls…". When using historical control data, it should be from the same timeframe for the exact strain, preferably from the same laboratory or the same supplier and preferably with the same pathologist[17]. This was not the case for the historical control database used by BfR. One of the mouse studies[8] was clearly done before this historical control database was developed, one study[14] used Crj:CD-1 mice rather than Crl:CD-1 mice, and 1 study[10] did not specify the substrain and was reported in 1993 (probably started prior to 1988); hence only a single study[16] used the right strain, but was reported more than 10 years after the historical control dataset was developed. Interestingly, the historical control data used by the BfR[21] was from studies in 7 laboratories using the Charles River Laboratory CD1 mice. Surprisingly, there is a second report[22] by the same authors with a larger control database using the same mouse strain from 11 laboratories over the same time period (1987-2000) showing very different results. For example, the 2000 publication[21] shows 5 and 4 studies out of 46 with adenomas and adenocarcinomas respectively whereas the 2005 report[22] shows only 1 study each out of 54 with a single adenoma and a single adenocarcinoma; all other studies had no tumors. Finally, in one mouse study[16] with malignant lymphomas, the comparison of the high exposure group to the control using four tests as reported by the BfR (Table 3-7) yielded p-values of 0.022, 0.022, 0.056 and 0.067; it is hard to see how this comparison to control can be disregarded.

Mechanistic Information

The BfR Addendum dismisses the WG finding that "there is strong evidence that glyphosate causes genotoxicity" by suggesting that the evidence we were not allowed to see was overwhelmingly negative and that, since the studies we did review were not done under guideline principles, they should get less weight. No consideration is given to the different strains and cell lines studied in the literature when compared to the guideline studies; no consideration is given to the different endpoints provided in the literature data; and the human in-vivo evidence is completely ignored. Because we are not able to evaluate the data that is proprietary, we are unable to comment on the veracity of their claim, but will note that, like the animal carcinogenicity data, the evaluations (Tables 4.2.1 to 4.2.7) seem to be simple and possibly miss dose-response trends.

The BfR confirms (p. 79) that the studies evaluated by the IARC WG on oxidative stress were predominantly positive but do not agree that this is strong support for an oxidative stress mechanism. They reduce the significance of these findings predominantly because of a lack of positive controls and because many of the studies used glyphosate formulations and not pure glyphosate. The WG disagrees with the BfR. We concluded that (p. 77) "Strong evidence exists that glyphosate, AMPA and glyphosate-based formulations can induce oxidative stress". Hence,

based on the studies we reviewed, not only were we able to identify glyphosate as inducing oxidative stress, but the formulations and AMPA as well.

Summary

The IARC WG concluded that glyphosate is a "probable human carcinogen" putting it into IARC category 2A. In their 2013 Draft RAR, BfR concluded (Vol. 1, p. 139) "classification and labelling for carcinogenesis is not warranted" and "glyphosate is devoid of genotoxic potential". How is this possible? Let's review the evidence and the conclusions.

The IARC WG saw an association between NHL and glyphosate in the human evidence, but could not rule out chance, bias and confounding; the IARC definition of "limited evidence"[1] for epidemiological data. BfR agreed, noting that other IARC categories are "not suitable". However the BfR concluded that an association was seen but dismissed it as insufficiently consistent.

The IARC WG saw significant effects for two tumors in two mouse studies and benign tumors in two rat studies. The BfR confirmed the statistically significant findings by the IARC WG, and agreed that the IARC criteria of "sufficient" evidence in animals is "fully met". BfR went on to identify two more mouse studies (bringing it to 3) with kidney tumors and another mouse study (bringing it to 2) with an increase in hemangiosarcoma, and two mouse studies showing increases in malignant lymphoma. Thus, all five mouse studies examined by the BfR were positive in at least 1 tumor site, 1 was positive in 3 tumor sites. Then using an inappropriate historical control dataset in an inappropriate way, dismiss all of these findings as chance.

The IARC WG concluded strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available data. The BfR, while confirming the positive studies we saw for genotoxicity dismissed them because they were not guideline studies and because, in their interpretation, all of the guideline assays were negative. The BfR confirmed the positive studies we saw for oxidative stress, noted some concern over these data, but concluded they could not use them because there were no other data to support a finding of carcinogenicity or genotoxicity and the mechanism cannot stand alone.

The basis of the IARC evaluation was the "limited evidence" in humans and the "sufficient evidence" in animals, conclusions the BfR note are consistent with the IARC criteria, with supporting evidence of 2 strong mechanisms. Is glyphosate the agent causing this hazard? Given the human evidence (co-formulants only), the animal evidence (glyphosate only) and the mechanistic evidence (all forms), the most logical scientific conclusion is that glyphosate is the probable carcinogen. The BfR dismissed all evidence in humans, dismissed all evidence in animals and concluded there was one weak mechanism that could not be used in isolation.

We feel that the process used by the BfR to review human and animal evidence is fundamentally flawed and should be reconsidered. We are of the opinion that the scientific basis for rejecting the human, animal and mechanistic studies is non-existent and appears to have been designed to achieve a pre-determined goal rather than an objective scientific review. Finally, we strongly object to the almost non-existent weight given to studies from the literature by the BfR and the strong reliance on non-publicly available data in a limited set of assays that define the minimum data necessary for the approval of a pesticide.

We stand by our conclusion that glyphosate is a probable human carcinogen.

1. IARC, *PREAMBLE TO THE IARC MONOGRAPHS* I. Monographs, Editor. 2006: Lyon, France. p. 25.
2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, *Glyphosate*, in *IARC Monogr Eval Carcinog Risks Hum*, I.M. Program, Editor. 2015. p. 1-92.
3. German Federal Institute for Risk Assessment, *Assessment of IARC Monographies Volume 112 (2015): Glyphosate*. 2015.
4. German Federal Institute for Risk Assessment, *Renewal Assessment Report*. 2013.
5. Das Erste. *BfRBewertung zu Glyphosat*. 2015; Available from: http://www.mdr.de/fakt/fakt-glyphosat-bfr-bewertung100.html.
6. Checkoway, H., N. Pearce, and D. Kriebel, *Research methods in occupational epidemiology*. 2nd ed. Monographs in epidemiology and biostatistics. 2004, New York: Oxford University Press. xiv, 372 p.
7. Schinasi, L. and M.E. Leon, *Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis.* Int J Environ Res Public Health, 2014. **11**(4): p. 4449-527.
8. Epa, *Glyphosate; EPA Reg. # 524-308; mouse oncogenicity study*, B. William Dykstra.Toxicology, Editor. 1985.
9. Chandra, M. and C.H. Frith, *Spontaneous renal lesions in CD-1 and B6C3F1 mice.* Exp Toxicol Pathol, 1994. **46**(3): p. 189-98.
10. JCFA, *Evaluation of certain food additives and contaminants: Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives*. 1999, Joint Committee on Food Additives (including C. Portier), World Health Organization/Food and Agriculture Organization: Geneva. p. 96.
11. Epa, *Second peer review of Glyphosate*. 1991. p. 1-19.
12. Epa, *Glyphosate - EPA Registration No. 524-308 - 2-Year Chronic Feeding/Oncogenicity Study in Rats with Technical Glyphosate*, I. William Dykstra.Toxicology Branch, Editor. 1991.
13. Epa, *Glyphosate; 2-Year Combined Chronic Toxicity/ Carcinogenicity Study in Sprague-Dawley Rats - List A Pesticide for Reregistration*, B. William Dykstra.Toxicology, Editor. 1991. p. 1-29.
14. Sugimoto, *18-Month Oral Oncogenicity Study in Mice.* Unpublished, designated

ASB2012-11493 in BfR RAR, 1997.
15. Unknown, *A chronic feeding study of glyphosate (roundup technical) in mice.* unpublished, designated ABS2012-11491 in BfR RAR, 2001.
16. Unknown, *Glyphosate Technical: Dietary Carcinogencity Study in the Mouse.* Unpublished, designated ABS2012-11492 in BfR RAR, 2009.
17. OECD, *Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies*, H.a.S.P. Environment, Editor. 2012, OECD: Paris.
18. Keenan, C., et al., *Best practices for use of historical control data of proliferative rodent lesions.* Toxicol Pathol, 2009. **37**(5): p. 679-93.
19. Haseman, J.K., G.A. Boorman, and J. Huff, *Value of historical control data and other issues related to the evaluation of long-term rodent carcinogenicity studies.* Toxicol Pathol, 1997. **25**(5): p. 524-7.
20. Greim, H., et al., *Evaluation of historical control data in carcinogenicity studies.* Hum Exp Toxicol, 2003. **22**(10): p. 541-9.
21. Giknis, M. and C. Clifford, *Spontaneous Neoplastic Lesions in the CrI:CD-1(ICR)BR Mouse*. 2000, Charles River Laboratories.
22. Giknis, M. and C. Clifford, *Spontaneous Neoplastic Lesions in the CrI:CD-1(ICR)BR Mouse in Control Groups from 18 Month to 2 year Studies*. 2005, Charles River Laboratories.